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The promastigote surface protease of *Leishmania donovani infantum* in the midgut of *Phlebotomus perniciosus*

Short communication

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Promastigotes of all *Leishmania* species tested up to the present display a major surface glycoprotein (63 kDa, gp63) of similar properties (Etges et al., 1985; Colomer-Gould et al., 1985). In 3 Old World species, *L. major, L. donovani* and *L. tropica,* there is strong evidence that these proteins are structurally related (Etges et al., 1985). In *L. major,* gp63 is present at 500,000 copies/cell which represents 1% of the total protein content of the entire cell (Bouvier et al., 1985). Recently gp63 has been identified as a protease and named promastigote surface protease, or PSP (Etges et al., 1986).

All these experiments were carried out using promastigotes cultivated in vitro. With the help of monoclonal antibodies directed against unrelated proteins (42 kDa and 90 kDa), surface epitopes of culture forms have been identified on promastigotes of *Leishmania mexicana amazonensis* proliferating in the midgut of *Lutzomyia longipalpis* (McMahon Pratt et al., 1983). The aim of our experiment was to demonstrate the presence of PSP on the surface of promastigote midgut-forms of *Leishmania donovani infantum* LEM288 isolated from the gut of *Phlebotomus perniciosus* Newstead, 1911.

Amastigotes of *L.d. infantum* LEM288 were produced by infecting hamster peritoneal macrophages with stationary-phase (promastigote) culture forms. The infected macrophages were offered together with washed packed human erythrocytes to 5–7 day old females of a laboratory colony of *Phlebotomus perniciosus* through a chicken-crop membrane at 33°C, using an artificial feeding device. The amastigote concentration was approx. 2×10^6/ml. 7 days after the infective meal, the guts of blood-fed females were dissected and microscopically examined. At this time, the bloodmeal was completely digested and the infection-rate was 98% (40/41 individuals). Promastigotes from heavily infected midguts (26/40) were transferred to drops of PBS (phosphate buffered

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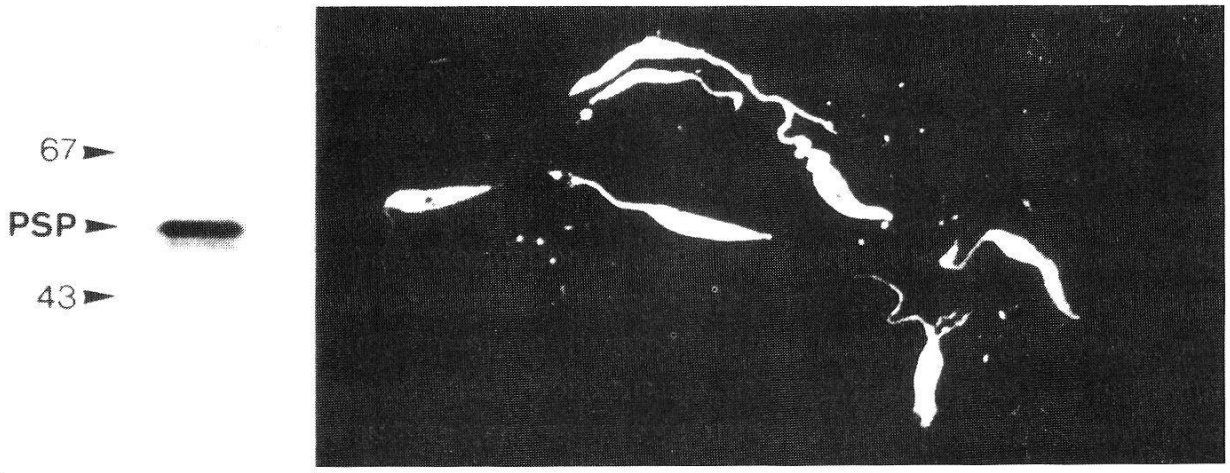

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Fig. 1. Western blot analysis of affinity purified anti promastigote surface protease antibody on an unreduced total protein extract of *Leishmania donovani infantum* LEM75 culture form promastigotes. One major protein (PSP) is recognised. The relative migration of reduced serum albumin (67 kDa) and ovalbumin (43 kDa) is indicated on the left.

Fig. 2. Indirect immunofluorescence assay on isolated promastigotes of *Leishmania donovani infantum* LEM288 from *Phlebotomus perniciosus* intestine, using the same antibody as in Fig. 1, at a final concentration of 220 ng/ml.

saline, pH 7.2) on marked positions of glass slides for the indirect immunofluorescent antibody test (IFAT). The slides were air dried at room temperature before storage at –70° C. The surface protease was demonstrated on the surface of the parasites by IFAT using an antibody that was prepared by immunization of New Zealand rabbits with purified, chemically deglycosylated *L. major* PSP (Bouvier et al., 1985), followed by affinity-purification on purified amphiphilic PSP bound to AffiGel 15, according to a protocol provided by the manufacturer. The specificity and inter-species crossreactivity of the antibody is shown by Western blot analysis of a crude extract of *L. d. infantum* (LEM75) proteins (Fig. 1). Two closely migrating forms of PSP are recognised by the antibody. The IFAT was performed on acetone-fixed promastigotes by two-fold antibody dilutions in PBS (pH 7.2) starting at 2 μg/ml. The goat anti-rabbit antibodies conjugated with FITC (fluorescein-isothiocyanate, IgG, Miles) were used at a 1:40 dilution in PBS containing Evans blue (1:10,000). Preimmune serum of the same rabbit and conjugated antibodies only were used as controls.

The isolated promastigotes from sandflies gave a positive reaction (Fig.2) down to an antibody concentration of 55 ng/ml. A distinct fluorescence activity covering the entire surface of the isolated promastigotes including the flagellum was seen in nearly all parasites (approx. 95%). No differences could be seen between single and dividing forms. Stationary-phase promastigotes cultured in vitro, which had been used for macrophage infection, also showed positive reactions. However, the proportion of these positive forms was lower and reached approx. 70%. All controls were negative.

These results show that PSP is present on the surface of the insect forms of *L. d. infantum* LEM288 after transformation from the amastigote to the promastigote form in the sandfly.

Evidence, that PSP is involved in the attachment of promastigotes to macrophages (Russel and Wilhelm, 1986), suggests that this protein is one of the factors important for the infectivity of *Leishmania* parasites. Sacks and Perkins (1984) showed that differences in infectivity occur during cultivation in vitro of different *Leishmania* parasites as well as during the development in the midgut of their insect vector, where infective stage promastigotes can be detected as early as 3 days after the infective blood meal (Sacks and Perkins, 1985). However, the recent finding that this protein is a protease allows new speculations regarding its function during the life cycle of *Leishmania* parasites (Etges et al., 1986).

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